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POSITION DOCUMENT NO. 1
ON BHC

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POSITION DOCUMENT NO. 1 ON BHC

I. BACKGROUND

A. Chemical/Physical Properties

Benzene hexachloride (BHC) has the empirical formula $C_6H_6Cl_6$. The commercial product, Technical BHC, is a mixture of several isomers, primarily alpha, beta, gamma and delta, which are present in the following proportions:

alpha-isomer	53 - 70%
beta-isomer	3 - 14%
gamma-isomer	11 - 18%
delta-isomer	6 - 10%

Three to five percent of the mixture is comprised of the following compounds:

Heptachlorocyclohexane	3 - 4%
Octachlorocyclohexane	0.5 - 1%
Oily compounds	0.5 - 3%

Technical BHC which has been enriched so that the gamma isomer is present at a level of 40 to 45% is marketed as fortified BHC. This document deals with technical BHC and all of the isomers present therein, including

the gamma isomer.[1/] When used without qualification, the term "BHC" will refer to technical BHC.

BHC is a relatively stable compound. Impurities such as pentachlorocyclohexene and tetrachlorocychlohexadiene give BHC its characteristic odor.

B. Environmental Characteristics

The fate of BHC in soil depends on environmental variables such as pH, moisture content, amount of organic material, temperature, and climatic conditions. BHC is removed from soil by volatization, chemical degradation and bacterial decomposition (Appendix A); it is non-persistent relative to other chlorinated pesticides, and soil residues are minimal (Appendix B). BHC has been detected in some aquatic segments of the environment, usually in concentrations of a few parts per billion (Appendix C). Gamma-BHC, the most water soluble of the chlorinated hydrocarbon pesticides, is soluble in water at approximately 10 ppm, although residues of this magnitude have not been reported.

In 1976, the Fish and Wildlife Service began participating in the National Pesticide Monitoring Program. This program is designed to assess various segments of the

1/ The gamma isomer of BHC is reported to be the most insecticidally-active isomer; gamma-BHC, 99% minimum purity, is marketed as the pesticide, lindane. Lindane is also being reviewed by the Office of Pesticide Programs and will be discussed in a separate document.

environment in order to locate areas where excessive pesticide residue levels are present or an increase of contaminants is noted from year to year. Although gamma-BHC has been found frequently in fish, no attempt has been made to determine whether its source was lindane and/or BHC (Appendix D).

The National Water Monitoring Program has detected gamma-BHC only once in unfiltered samples since February 1975. A sample from Hawaii contained residues of 0.01 ug/l. Approximately 850 samples of water and 425 samples of bottom sediment collected from sites throughout the nation were analyzed for gamma-BHC (Appendix C).

From October 1968 to July 1970, shellfish samples from ten estuaries in Long Island, N.Y., were collected monthly and examined for chlorinated hydrocarbons as part of a continuing nationwide survey conducted by the National Marine Fisheries Service, U.S. Departmernt of Commerce. Gamma-BHC was the only isomer monitored, but it was not detected in any of the samples (Appendix E).

Organochlorine pesticides were monitored nationwide in the wings of more than 24,000 mallards and black ducks bagged during the 1965 and 1966 hunting seasons. Gamma-BHC was found in trace amounts in wings of birds from Washington and Michigan.

Monitoring data collected by the National Human Monitoring Program show that BHC is consistently found in human adipose tissue. Analysis of samples collected throughout the 48 coterminous States for fiscal years 1970, 1971, and 1972 shows that beta-BHC occurred in human adipose tissue at a frequency of 99.15, 99.32, and 99.22%, respectively, with a geometric mean concentration of 0.37, 0.34 and 0.23 ppm. Alpha-BHC occurred in 2.05% of 1,412 samples analyzed for fiscal year 1970 (Appendix F).

Alpha-BHC was found in 87.37% of ambient air samples from 14 to 16 selected States during the years 1970 to 1972. The arithmetic mean concentration of 2,479 samples was 1.1 ng/m^3 (Appendix G). The percentages of positive samples for other chlorinated hydrocarbons ranged from a low of 0.08% for chlordane to a high of 98.22% for p,p' DDT. The arithmetic mean concentration varied from 0.1 ng/m^3 for chlordane to 5.6 ng/m^3 for p,p' DDT.

C. Registered Uses

Since the early 1950's, BHC has been registered for foliar and soil applications to fruit and vegetable crops, ornamentals, lawn, turf, and forests. BHC has also been registered in dips, sprays or dust formulations for use on

horses, mules and dogs. It is also used in preparations for the control of human parasites. Currently, there are 65 Federal registrations for BHC distributed among 45 registrants. In addition, there are 18 intrastate registrations distributed among 15 registrants.

BHC is formulated primarily as wettable powders, emulsifiable concentrates, dusts, and, in some cases, liquids. Production data are confidential and are therefore not a part of this document.

Hooker Chemicals and Plastics Corporation of Niagara Falls, New York, is the principal domestic producer of BHC. According to Dr. Richard S. Goldman of Hooker, all production facilities for BHC at the Hooker plant were shut down in May 1976.

On September 1, 1976, Hooker requested a voluntary cancellation of its BHC registrations under the provisions of section 6(a)(1) of the Federal Insecticide, Fungicide, and Rodenticide Act (1975). Notice of voluntary cancellation will be published simultaneously with the Notice of Rebuttable Presumption Against Registration accompanying this document (Appendix H).

II. REGULATORY HISTORY

In 1969 the Report of the (HEW) Secretary's Commission on Pesticides and Their Relationship to Environmental Health ("Mrak Commission") listed BHC among several pesticides which "are persistent and cause or can cause contamination of the environment and damage to various life forms within it (Appendix I)." The Commission recommended that the use of these persistent pesticides should be restricted "to specific essential uses which create no known hazard to human health or to the quality of the environment (Recommendation 4, p. 9)."

On March 18, 1971 the EPA Administrator announced that active internal review was being initiated of registration of products containing BHC, lindane and certain other pesticides "to identify which, if any, of the presently registered products presented substantial questions of safety that should trigger the administrative process of cancellation."

In accordance with the Administrator's Announcement, the Special Pesticide Review Group, OPP, reviewed in 1973 the hazards associated with the use of BHC (Appendix J) and prepared a memorandum recommending cancellation of BHC registrations (Appendix K). A draft notice of intent to cancel was prepared February 15, 1975 but was not issued (Appendix L).

A working group with representatives from all relevant Divisions and Offices of EPA was formed to prepare this Position Document. Dr. Albert, as Chairman of the Carcinogen Assessment Group (CAG) was asked to evaluate the data on oncogenicity; and the Validation Committee of Criteria and Evaluation Division was asked to review chronic data. Drs. Chernoff and Courtney, Research Triangle Park, reviewed fetotoxic and reproductive data.

Tolerances of 0.01 to 1 ppm are in effect for BHC on harvested residues for a variety of crops. Some of the original tolerances were reduced primarily to bring them into line with the policy that the residue should be no higher than the amount reasonably expected to result when label directions for use are followed (Appendix M).

III. SUMMARY OF SCIENTIFIC EVIDENCE BY REBUTTABLE PRESUMPTION CRITERIA

A. Chronic Toxicity

1. Oncogenic Effects in Test Animals

The regulations in 40 CFR Section 162.11(a)(ii)(A) include the following statement: "A rebuttable presumption shall arise if a pesticide's ingredient(s). . . (i)nduces oncogenic effects in experimental mammalian species or in

man as a result of oral, inhalation or dermal exposure . . . ."[2/] The working group is aware of several feeding studies involving BHC and various BHC isomers which meet this criterion. The studies have been reviewed by the CAG and the CED Validation Committee.

The CED Validation Committee reviewed the laboratory animal feeding studies by Thorpe and Walker, Herbst et al., Goto et al., and Nagasaki et al. (Appendix N) and concluded that none of these studies presented data adequate to meet or exceed the risk criteria for an RPAR. For example, all the studies were limited to mice and the only tumor type found to increase at high dosages was hepatoma. In the Thorpe and Walker study, approximately 10% of the males and 20% of the females receiving 400 ppm gamma-BHC died during the first three months. These results suggested to the committee that the 400 ppm dose may be higher than the maximum tolerable dosage and that the liver enlargement and the hepatoma observed in the animals may have resulted from nonspecific toxic effects of the materials largely caused by excessive workload on the liver (Appendix N).

2/ The term "oncogenic" is defined in 40 CFR Section 162.3(bb) as "the property of a substance or a mixture of substances to produce or induce benign or malignant tumor formations in living animals."

The International Agency for Research on Cancer Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Man met in Lyons on October 22-29, 1973. They concluded that BHC, its pure alpha, beta, and gamma isomers, and the mixture of delta plus epsilon isomers are carcinogenic in mice, producing liver-cell tumors following oral administration. This group also concluded that available feeding studies in rats were considered inadequate either because survival rates were low, the information reported was insufficient, or the doses were too low. The studies cited in support of IARC's conclusion are some of the same ones reviewed by the CED Validation Committee and CAG.

CAG has recently reviewed these and several other feeding studies in which (BHC) was evaluated (Appendix DD). According to CAG, these studies indicate that BHC and various isomers of BHC induce statistically significant oncogenic effects in both mice and rats as a result of oral exposure. BHC or its isomers have been found to be oncogenic in seven strains of mice, although the degree of susceptibility to tumor induction differs among the various strains. The feeding studies have also revealed a higher susceptibility in males than in females. In addition, there was an increased incidence of tumors produced by both the alpha and beta isomers of BHC when combined with polychlorinated biphenyls (PCBs).

A brief summary of the studies indicating oncogenic effects is given below, together with other studies which were negative or inconclusive.

(a) Nagasaki et al. (1973), Mouse Study - BHC.

Nagasaki et al. (1973) observed that 660 ppm of BHC in the diet of male dd strain mice for 24 weeks induced carcinomas and hyperplastic nodules in the liver (Appendix O). Sixty male dd strain mice (three groups of 20) were fed diets containing 660,[3/] 66, or 6.6 ppm BHC. Fourteen male dd mice were used as controls. Hepatomas were induced in 100% (20) of those mice fed 660 ppm BHC. The results were statistically significant at $p<10^{-10}$. Hepatomas observed were described as histologically similar to hepatomas induced by the following chemical carcinogens: o-aminoazotohene, 4-dimethylaminoazobenzene, N-nitrosodimethylamine, and N-nitrosopiperidine. No hepatomas were observed in the two groups fed 6.6 or 66 ppm BHC; however, cellular hyperplasia was observed in the group fed 66 ppm and, to a lesser extent, in that fed 6.6 ppm. An increase in liver weight was observed in all treated groups; livers in the mice fed 660 ppm increased 300% over the liver weights of the control

3/ The amount of each isomer in the 660 ppm BHC dose was as follows: 439 ppm alpha-BHC, 75 ppm beta-BHC, 100 ppm gama-BHC, 42 ppm delta-BHC, and 4 ppm other isomers.

animals. There was also some effect on the spleen weight. These observations, as well as those on BHC intake and its accumulation in the liver, are summarized in Tables 1 and 2. No hepatomas or nodular hyperplasia were observed in the control group.

TABLE 1. EFFECTS ON LIVER AND SPLEEN WEIGHTS IN MICE FED BHC FOR 24 WEEKS

Dosage (ppm)	No. per Group	Liver (g)	Spleen (g)
600	20	5 ± 1.8	0.13 ± 0.06
66	20	2 ± 0.3	0.19 ± 0.09
6.6	20	1.9 ± 0.7	0.26 ± 0.14
0	14	1.6 ± 0.3	0.24 ± 0.16

TABLE 2. INTAKE OF BHC AND ITS ACCUMULATION IN THE LIVER AFTER 24 WEEKS

Dosage (ppm)	Total intake (mg/mouse)	Accumulation of isomer (ppm)	
		Alpha	Beta
660	555.21	11.44	12.37
66	59.06	1.13	1.36
6.6	6.20	0.22	0.23
0	0	.01	.01

(b) Hanada et al. (1973), House Study - BHC.

Hanada et al. (1973) separated six-week-old male and female dd strain mice into groups and fed them diets containing 0, 100, 300, or 600 ppm BHC for 32 weeks, followed by a basal diet for 5 to 6 weeks (Appendix P). 4/ Hepatomas observed in both males and females fed 600 or 300 ppm were histologically similar to hepatomas induced in mice by CCl_4 and azo dye. In the group fed 600 ppm, 100% of the animals (4 males and 5 females) developed hepatomas. The average size of liver tumors in the 600 ppm group was 11 mm for males (which was the largest average size of any feeding or sex group in the study) and 9 mm for females. In animals fed 300 ppm, all four male mice and three of the five females developed hepatomas. The average size of the liver tumors in these animals was 6 mm for males and 5 mm for females. No hepatomas were observed in animals fed 100 or 0 ppm. These results are statistically significant ($p<0.001$).

(c) Goto et al. (1972), Mouse Study - BHC.

Goto et al. (1972). reported the results of a study in which BHC was fed to IRC-JCL male mice (Appendix Q). Results confirm the observations reported by other researchers

4/ In addition, male and female groups of mice were fed diets containing alpha, beta, or gamma BHC at 100,300, and 600 ppm. The oncogenic effects caused by the individual isomers are shown in Table 5.

that BHC is oncogenic. Five-week-old male IRC-JCL mice were fed a diet containing 600 ppm BHC. After 26 weeks, ten of the mice were sacrificed and examined for gross and histological changes. Hepatomas were observed in all of the animals and, although the exact numbers were not reported, some of the heptomas were characterized as being malignant ($p<0.0001$). In addition, the liver weight of the animals fed diets containing BHC was almost four times greater than that of the control animals. No hepatomas were observed in the control animals.

(d) Nagasaki et al. (1972), Mouse Study - Alpha-, Beta-, Gamma-, or Delta-BHC.

Nagasaki et al. (1972), separated 260 male dd strain mice into groups of 20 mice each and fed them diets containing 500, 250, 100 or 0 ppm of either alpha, beta, gamma, or delta BHC for 24 weeks (Appendix R). Only the animals fed diets containing 500 or 250 ppm alpha-BHC developed hepatic carcinomas. Of those fed 500 ppm alpha-BHC, 65% (13 out of 20) developed hepatocellular carcinomas; 35% (7 out of 20) developed nodular hyperplasia. Of those fed 250 ppm alpha-BHC, 15% (3 out of 20) developed hepatocellular carcinomas and 70% (14 out of 20) developed nodular hyperplasia. Hepatic cell hypertrophy of varying degrees was observed in most of the other groups. All of these results are statistically significant ($p<0.0001$).

(e) Ito et al. (1973), Mouse Study - Alpha-, Beta-, Gamma-, or Delta-BHC.

Ito et al. (1973) fed groups of 20 to 40 8-week-old male dd strain mice diets containing 500, 250, 100, or 0 ppm alpha-, beta-, gamma-, or delta-BHC plus combinations of the various isomers (Appendix S). The combinations were as follows: 250 ppm alpha- with either 250 ppm beta-, 250 ppm gamma-, or 250 delta-BHC; 250 ppm beta-BHC with either 250 ppm gamma- or 250 ppm delta-, and 250 ppm gamma- plus 250 ppm delta-BHC. Hepatocellular carcinomas, nodular hyperplasia and hypertrophy of centrolobular liver cells developed in all groups receiving alpha-BHC either alone or in combination with another isomer, except for the group fed 100 ppm alpha-BHC. Although animals fed diets for 24 weeks containing 250 ppm beta-BHC or 250 ppm gamma-BHC did not develop hepatic lesions, animals fed diets containing 250 ppm alpha- plus either the 250 ppm beta- or 250 ppm gamma-BHC had a greater incidence of both nodular hyperplasia and hepatocellular carcinomas than had animals fed diets containing 250 ppm alpha-BHC (Table 3). No hepatic nodules or other effects were observed in the control group. All of these results are statistically significant ($p<10^{-7}$).

TABLE 3. HISTOPATHOLOGICAL CHANGES IN THE LIVER OF MICE TREATED WITH BHC ISOMERS FOR 24 WEEKS

Isomer in diet (ppm)	No. per Group	Nodular hyperplasia total	Nodular hyperplasia %	Hepatocellular carcinoma total	Hepatocellular carcinoma %
alpha 500	20	20	100.0	17	85.0
alpha 250	38	30	78.9	10	26.3
alpha + beta 250 + 250	28	26	92.8	14	50.0
Alpha + gamma 250 + 250	28	26	92.8	12	42.8
alpha + delta 250 + 250	28	21	75.0	7	25.0
Control 0	20	0		0	

(f) Goto et al. (1972), Mouse Study - Alpha-, Beta-, or Delta-BHC.

In addition to studying the oncogenic effects of BHC, Goto et al. (1972) studied the oncogenic effects of the isomers of BHC (Appendix Q). Liver tumors and an increase in liver weight were observed in all animals fed 600 ppm of the various BHC isomers for 26 weeks (Table 4).

TABLE 4. EFFECTS OF VARIOUS ISOMERS ON ICR-JCL MALE MICE LIVERS

BHC isomer	PPM	No. per Group	Liver weight (g)	Liver Tumors total	Liver Tumors %
Alpha	600	10	9.9	10	100
Beta	600	10	4.8	*	
Gamma	600	10	4.1	5	50
Delta & Epsilon	600 (total)	10	4.4	8	80
Control		10	2.4	0	0

*Tumors were observed, but numbers were not reported.

In all mice fed the treated diet there was atypical proliferation of cells and benign neoplasms. In addition, diets containing 600 ppm alpha-BHC and 600 ppm of delta-plus epsilon-BHC induced frequent malignant neoplasms which appeared similar to human liver cancer. The three cases for which numerical values are given are significantly different from the controls ($p<0.0001$).

(g) Hanada et al. (1973), Mouse Study - Alpha-, Gamma-BHC.

Hanada et al. (1973) investigated the oncogenic activity of alpha- and gamma-BHC in male and female dd strain mice (Appendix P). In both females and males that survived, the incidence of tumors was higher than in controls (Table 5).

TABLE 5. TUMOR INCIDENCE IN MALE AND FEMALE dd STRAIN MICE

Isomer	PPM	Males		Females	
		Total males	Total tumors	Total females	Total tumors
Alpha-BHC	100	8	1	8	0
	300	7	7	3	2
	600	7	7	8	6
Gamma-BHC	100	10	0	8	0
	300	9	0	7	0
	600	4	3	3	1
Control	0	11	0	15	0

An oncogenic effect in both female and male mice was observed. Moreover, hepatic changes in males were more pronounced than in females. For alpha-BHC, the results are significant for males at $p<.0001$ and for females at $p<.001$. For gamma-BHC, the results are significant for males at $p<0.025$ and not significant for females.

(h) Thorpe and Walker (1973), Mouse Study (Tunstall Laboratory) - Gamma- or Beta-BHC.

In 1973, Thorpe and Walker (Appendix T) of the Tunstall Laboratory (Shell Research Limited) conducted a study using the beta- and gamma-isomers as part of a larger study on the pesticides dieldrin and DDT. The results indicated that both beta- and gamma-BHC induced a statistically significant increase in hepatic tumors for males ($p<.0001$ for both isomers) and females ($p<.05$ for beta-BHC and $p<.0001$ for gamma-BHC). In addition, both the beta and gamma isomers increased the incidence of "type b" tumors which the researchers characterized as malignant. The isomers also increased the incidence of metastases to the lung. Researchers did not conduct serial sections of the lung so that invasion or metastasis could be detected.

In the study, Carworth Farm No. 1 (CF1) 14-week-old male and female mice were fed diets for up to 110 weeks containing 200 ppm beta-BHC, 400 ppm gamma-BHC, or 0 ppm. The mortality level was high. Only 17% of the males and 3% of the females fed diets containing gamma-BHC survived for the full 110 weeks. Thirteen percent of the males and 17% of the females fed diets containing beta-BHC survived for the duration of the study. Forty-four percent of the male control animals and 32% of the female control animals survived for the full term. Livers were enlarged by the fiftieth week in both males and females fed diets containing 200 ppm beta-BHC and by the sixtieth week in males fed 200 ppm beta-BHC. The first liver tumor was found at autopsy in a female fed 40 ppm gamma-BHC for 12 months. Researchers classified the tumors "type a" (which did not have the capacity to metastasize) and "type b" (which did have the capacity to metastasize). The specific incidence of each type of liver tumor observed in the males and females is presented in Tables 6 and 7. Beta-BHC and gamma-BHC not only increased the incidence of tumors of the liver in CF1 mice, but also resulted in more "type b" hepatic tumors, considered by the authors to be

malignant tumors. There was also more metastasis to the lung. Treated mice developed hepatic tumors earlier and died sooner than did the controls. Dr. Melvin Reuber, CAG, reported that the "type a" and "type b" tumor classifications of liver in mice were arbitrary and differed from published descriptions of the morphologic and biologic behavior of liver lesions in mice and rats. He evaluated the "type a " and "type b" tumor classifications by obtaining and examining the histologic sections from the dieldrin-DDT subexperiment by the Tunstall Laboratory. He reported that carcinomas with one histologic pattern are called "type b" tumors and are considered to be malignant because they can metastasize to the lungs. All other lesions of parenchyma cells of the liver are grouped under "type a" benign tumors. "Type a" tumors, therefore, can include hyperplastic nodules of the liver as well as carcinomas of the liver. Some of the "type a" tumors have the morphologic characteristics of malignant tumors, metastasize to the lungs, and grow following transplantation in other mice of the same strain. This classification decreases the incidence of carcinomas of the liver because some carcinomas are improperly included under benign "type a" tumors. In control mice, the earliest tumors of the liver were found in a male that died at 18 months and a female that died at 23 months.

Exposure to beta-BHC or gamma-BHC was associated with a statistically significant increased incidence of hepatic lesions. The p value was $p<0.0001$ for males and females ingesting gamma-BHC or beta-BHC. There was also a striking increase both in "type b" and "type a" tumors in the treated mice when compared to the untreated controls. There were 17% lung metastases in male mice given beta-BHC and 11% in male mice give gamma-BHC, compared to 0% in the controls. These findings have been summarized in Tables 6 and 7.

The overall incidence of tumors other than for liver appeared to be reduced in most groups of treated animals. Generally, the treated mice died early with tumors of the liver. The nontreated controls died early with extrahepatic tumors and developed hepatic tumors late in the study. No mention was made as to the location of the extra hepatic tumors, or whether they were benign or malignant in treated mice. There is a possibility that these tumors were benign in controls and malignant in treated mice, as was the case for liver tumors (Tables 6 and 7).

TABLE 6. INCIDENCE OF TUMORS OF THE LIVER IN MALE MICE INGESTING BETA-BHC OR GAMMA-BHC FOR UP TO 110 WEEKS

Isomer	PPM	No. per Group*	Type a Tumors		Type b Tumors		Total Tumors	Lung Metastases	
			total	%	total	%	%	total	%
Control	0	45	9	20	2	4	24	0	0
B-BHC	200	24	12	50	10	42	92	4	17
ɣ-BHC	400	28	11	38	16	58	96	3	11

*Animals which died before the first tumors were detected are excluded.

TABLE 7. INCIDENCE OF TUMORS OF THE LIVER IN FEMALE MICE INGESTING BETA-BHC OR GAMMA-BHC FOR UP TO 110 WEEKS

Isomer	PPM	No. per Group*	Type a Tumors		Type b Tumors		Total Tumors	Lung Metastases	
			total	%	total	%	%	total	%
Control	0	44	10	23	0	0	23	0	0
B-BHC	200	19	9	47	4	21	68	0	0
ɣ-BHC	400	21	10	47.5	10	47.5	95	1	5

*Animals which died before the first tumors were detected are excluded.

(i) Nagasaki et al. (1975), Mouse Study - Alpha-BHC.

Nagasaki et al. (1975) investigated oncogenic effects of alpha-BHC in five different strains of male and female mice, 5- to 6-week-old male Wistar strain rats, and 5-week-old male golden Syrian hamsters. All animals were fed a diet containing 500 ppm alpha-BHC for 24 weeks (Appendix U). Differences in tumor incidence between strains of mice were observed; DDY mice had the highest incidence and C57BL/6 mice had the lowest. In the male mice, nodular hyperplasia were observed in all five strains, and hepatocellular carcinomas were observed in two strains (DDY and IRC) and, to a lesser extent, in a third (DBA/2). In female mice, the observations were basically the same as for males, but the effect was less pronounced. Neither nodular hyperplasia nor hepatocellular carcinomas were observed in the controls of any strain. The overall results are statistically significant ($p<0.0001$) and are shown in Table 8. Neither nodular hyperplasia nor hepatocellular carcinoma were observed in any rats or hamsters.

TABLE 8. LIVER EFFECTS IN MICE FED 500 PPM ALPHA-BHC FOR 24 WEEKS

Strain	Sex	No. per Group	Nodular Hyperplasia Total	Nodular Hyperplasia %	Hepatocellular Carcinomas Total	Hepatocellular Carcinomas %
DDY	M	20	20	100.0	13	65.0
	F	20	16	80.0	5	25.0
ICR	M	23	18	78.3	8	39.1
	F	29	15	51.7	6	20.0
DBA/2	M	16	8	50.0	1	6.3
	F	15	5	33.0	1	6.7
CF7BL/6	M	21	4	19.0	0	0
	F	18	3	16.7	0	0
C3H/He	M	20	13	65.0	0	0
	F	20	11	55.0	2	10.0
Control	M	*	0	0	0	0
	F	*	0	0	0	0

*Number of controls for each strain varied from 13 to 22.

(j) Ito et al. (1973), Mouse Study - BHC Isomers in Combination with Polychlorinated Biphenyls (PCBs).

Ito et al. (1973) investigated the effects of PCBs on the induction of tumors when combined with alpha-, beta-, or gamma-BHC (Appendix V). The results indicated

that PCB-5[5/] promotes tumor induction when combined with alpha-BHC and, to a lesser extent, beta-BHC, but does not promote tumor induction when combined with gamma-BHC.

In the study, 8-week-old male dd strain mice were first fed diets containing 100, 250, or 500 ppm PCB-3, -4, or -5 for 32 weeks. Only animals receiving 500 ppm PCB-5 developed nodular hyperplasia (7 out of 12, or 58.3%) and hepatocellular carcinoma (5 out of 12, or 41.7%); however, focal hypertrophy of the centrolobular liver cells was observed in all groups fed PCB-5 and PCB-4. Subsequently, additional male dd strain mice were fed diets ranging from 50 to 250 ppm (Table 9).

Hyperplastic nodules or hepatocellular carcinomas were not observed in any groups fed diets containing only alpha- or beta-BHC, with the exception of those mice fed 250 ppm alpha-BHC. In that group, 76.6% (23 out of 30) developed hyperplastic nodules, and 26.7% (8 out of 30 developed carcinomas. However, the addition of PCBs to the various dosages of alpha- and beta-BHC either increased the incidence of tumors or induced tumors in groups fed the alpha or beta isomers in amounts which previously had not induced observable tumors.

5/ PCB samples were Kanechlor-500, -400, and -300.

TABLE 9. LIVER NODULE INDUCTION IN MALE dd MICE FED BHC AND PCB-5

Treatment	PPM	No. per Group	Liver Nodules (%) Nodular Hyperplasia Total	Nodular Hyperplasia %	Hepatocellular Carcinoma Total	Hepatocellular Carcinoma %
alpha-BHC	250	30	23	76.7	8	26.7
alpha-BHC +PCB-5	250 250	26	21	80.8	15	57.6
alpha-BHC	100	26	0	-	0	-
alpha-BHC +PCB-5	100 250	25	8	32.0	1	4.0
alpha-BHC	50	28	0	-	0	-
alpha-BHC +PCB-5	50 250	30	9	30.0	2	6.7
beta-BHC	250	26	0	-	0	-
beta-BHC +PCB-5	250 250	29	16	55.2	16	55.2
beta-BHC	100	26	0	-	0	-
beta-BHC +PCB-5	100 250	30	5	16.7	1	3.3

(k) Ito et al. (1975), Rat and Hamster Study - Alpha-, Beta-, or Gamma-BHC Isomers.

Ito et al.(1975) studied male Wistar strain rats fed diets containing 500 ppm alpha-, beta-, or delta-BHC for 24 or 48 weeks; 1,000 ppm alpha-BHC for 24, 48, or 72 weeks; 1,000 ppm beta-BHC for 24 weeks; 1,000 ppm delta-BHC for 24 or 48 weeks; and 1,500 ppm alpha-BHC for 72 weeks (Appendix W).

Carcinomas of the liver were observed in only those rats given 1,500 or 1,000 ppm alpha-BHC for 72 weeks; 23% (3 out of 13) had carcinomas, and 77% (10 out of 13) had hyperplastic nodules. In those given 1,000 ppm alpha-BHC for 72 weeks, 6% (1 out of 16) had carcinomas of the liver, and 76% (12 out of 16) had hyperplastic nodules. Rats given 1,000 ppm alpha-BHC for 48 weeks developed only hyperplastic nodules; 5 out of 12 or 42% were so affected. Results for the hyperplastic nodules are statistically significant at $p<0.001$.

High dosages of beta-, gamma-, and delta-BHC fed for 24 or 48 weeks did not induce tumors but did induce cellular hypertrophy.

(l) Fitzhugh et al. (1950), Rat Study - Alpha-, Beta-, or BHC.

Fitzhugh et al. (1950) conducted a lifetime feeding study (approximately 110 weeks) with male Wistar strain rats (Appendix X). The animals were fed diets containing either alpha-, beta-, or technical BHC in amounts of 10 to 800 ppm.

Although no tumors were observed in either the control or treated groups, the authors reported microscopic alterations in the liver typical of those seen with other chlorinated compounds such as DDT, aldrin, and dieldrin. The histological alterations in the liver were centrolobular hypertrophy and diffuse hepatic cell hypertrophy, as well as hepatic cell atrophy, fatty degeneration, and focal necrosis. Slight to moderate kidney damage occurred in animals treated with 800 ppm alpha-BHC, and slight damage was seen in those treated with 800 ppm technical BHC. A moderate degree of testicular atrophy was observed in the group treated with 800 ppm technical BHC.

Livers were enlarged in all groups treated with all isomers and technical BHC. The lowest dosage which significantly affected liver weight was 10 ppm for beta-BHC and 100 ppm for alpha-, beta-, gamma-, or technical BHC (Table 10).

TABLE 10. LIVER WEIGHTS OF RATS FED DIETS CONTAINING BHC OR ITS ISOMERS

Dosage (ppm)	Mean weights (g/kg of body weight)					
	Control	Alpha	Beta	Gamma	Dry Gamma*	Technical
0	31.5					
5				29.4		
10		34.7	42.1	31.3	33.8	32.3
50		41.6		33.9		33.7
100		45.4	46.7	42.5	35.9	42.7
800		87.3	98.2	66.3	50.1	3.5
1600				76.3		

* Gamma-BHC without the corn oil vehicle.

Tissue storage of the various isomers was measured. The beta isomer was stored in greatest amount followed by the alpha- and gamma-isomers. In decreasing order of storage, the isomers were detected in fat, kidney, brain, muscle and liver tissues. The exact amounts for each isomer and tissue location are shown in Table 11.

TABLE 11. TISSUE STORAGE IN FEMALE RATS FED DIETS CONTAINING 100 OR 800 PPM OF BHC ISOMERS FOR ABOUT 20 MONTHS

Isomer	Dosage (ppm)	Stored Isomers (PPM) Fat	Kidney	Brain	Muscle	Liver
Alpha	100	120	70	70	30	20
Alpha	800	3,500	180	230	75	120
Beta	100	2,000	150	130	70	30
Beta	800	*	*	*	*	*
Gamma	100	120	80	30	20	0
Gamma	800	440	75	73	20	0

*No survivors.

The mean ages at death of rats fed diets containing BHC or various isomers of BHC are shown in Table 12. Rats fed diets containing the alpha and beta isomers died earlier than those on other regimens.

TABLE 12. MEAN AGE AT DEATH OF RATS FED DIETS CONTAINING BHC OR ITS ISOMERS

Dosage (ppm)	Mean age at death (weeks) Control	Alpha	Beta	Gamma	Dry Gamma*	Technical
0	58.3					
5				69.8		
10		54.6	52.9	60.5	56.4	
50		54.9		54.3		60.7
100		56.2	43.4	63.7	60.2	64.6
400				47.1		
800		35.9	4.4	39.7	57.6	32.9
1,600				38.2		

*Gamma-BHC without the corn oil vehicle.

Fitzhugh et al. (1950) did not observe any liver tumors in rats given a maximum of 800 ppm alpha-BHC whereas a dose of 1,500 ppm alpha-BHC was found by Ito et al. (1975) to induce liver tumors (Appendix W).

(m) Herbst et al. (1975), Mouse Study - Gamma-BHC

Herbst et al. (1975) fed 24-day-old NMRI male and female mice , 0, 12.5, 25 or 50 ppm gamma-BHC in the diet for 80 weeks. Fifty mice of each sex were used as the test animals. One hundred of each sex were controls. The author concluded that there was no increase in liver tumors. There was, however, leukemia in 6 out of 49 females ingesting 12.5 ppm lindane (12.2%) and 5 of 98 control females (5.1%).

This study used much lower dosages than those used in the other mouse studies. It would be necessary to review the histology of the liver to determine if centrolobular hyperplasia or hyperplastic nodules occur at these low doses (Appendix Y).

2. Reproductive and Fetotoxic Effects in Test Animals

40 CFR 162.11(a)(3)(ii)(B) provides that: "[a] rebuttable presumption shall arise if a pesticide's ingredient . . . . [p]roduces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as

determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety." The Working Group is aware of two studies, summarized below, which demonstrate that for two isomers of BHC there is sufficient evidence to establish a presumption pursuant to Section 162.11(a)(3)(ii)(B).

Yamagishi et al. (1972) reported that beta-BHC produced fetotoxic effects in mice (Appendix Z). In this study, fetotoxicity was evaluated by determining the relative proportions of intrauterine deaths and immature fetuses in control and beta-BHC treated mice. Immaturity was determined by subjective evaluation of size, motor activity and responsiveness. Resorbed and dead fetuses were counted as intrauterine deaths. Two of the experiments involved subcutaneous administration of 5, 50 or 100 µg/g beta-BHC during either early (days 2-6) or middle (days 7-11) gestation. In a third experiment, pregnant females which had been maintained on a diet containing 5 ppm beta-BHC for one month prior to mating were fed 50 or 100 µg/g beta-BHC on the sixth and seventh days of gestation. The rate of immaturity and death in the offspring are recorded in Table 13.

TABLE 13. INCIDENCE OF DEAD AND IMMATURE FETUSES FOLLOWING EXPOSURE TO BETA-BHC

Protocol	Percent Immature and Dead or Resorbed Fetuses			
	0 (control)	5 ug/g	50 ug/g	100 ug/g
Oral admin. of 5 ppm beta-BHC one month before mating plus oral doses on days 6 and 7	8% (9/112)	11.3% (14/124)	24.0% (30/126)	25.8% (31/120)
Subcutaneous admin. in early gestation (days 2-6)	23.6% (26/110)	23.8% (30/126)	35.6% (46/129)	47.37% (52/110)
Subcutaneous admin. in middle gestation (days 7 to 11)	20% (20/100)	30% (35/115)	24.6% (32/130)	21.3% (27/127)

The data suggest that exposure to 50 or 100 ug/g beta-BHC during the first half of the 21-day gestation period increases the incidence of fetal mortality and immaturity. The first protocol listed in the table is particularly significant since it tends to duplicate possible exposure patterns of humans who have residual tissue levels of beta-BHC and then receive a short-term high level exposure from normal use of the pesticide. However, since data is not available on the amount of human exposure which may occur during normal use, the question remains open as to whether the levels where effects were observed in the animals are substantially higher than those "to which humans can reasonably be anticipated to be exposed." Moreover, since beta-BHC is detected in the tissue of about 99% of all persons living in the United States, it must be presumed that current exposure may be sufficient to produce feto-toxic effects in humans.

This study and the Petreschu and Naishtein studies described below were reviewed by Drs. Chernoff and Courtney at RTP, and their comments were discussed with several members of the working group (Appendix AA).[6/] Both Drs. Chernoff and Courtney agreed that there were several limitations in the researchers' reporting of observed effects. The most important was the omission of specific fetal weight measurements, even though the primary parameter of fetal effects was the observation of fetal growth and development. Dr. Chernoff also noted that subcutaneous administration of the pesticide made any appraisal of the amount of the chemical exposure difficult. In addition, Dr. Chernoff expressed concern over the small number of animals studied and concluded that the results would have been more significant if more animals had been included in each group. Dr. Chernoff also commented on the variation in control mortality.

However, both scientists concluded that the first experiment (see Table 13) appeared valid for purposes of indicating that beta-BHC produces fetotoxic effects in mice, but that defective reporting of some experimental details reduced the weight which should have been given to the researchers' conclusions. In addition to the observed fetotoxic effects, Dr. Courtney noted that

6/Dr. Chernoff reviewed the Yamagishi study. Dr. Courtney reviewed the Yamagishi study as well as the Petreschu and Naishtein studies.

measurements of BHC levels in maternal and fetal tissue demonstrated that "beta-BHC crosses the placenta and is deposited in the fetus in a dose dependent manner."

Petreschu, et al. (1974) (Appendix BB) reported that lindane, the gamma-isomer of BHC, has an adverse effect on normal reproductive functions in the white rat. This study demonstrated that in rats fed diets containing 0, 5, 10, and 15 mg/kg gamma-BHC for three generations, the average duration of pregnancy lenghtened somewhat, and the fertility index (number of births relative to the size of the parental population) decreased. The study also reported that there was a progressive increase in the proportion of stillbirths with each successive generation. In addition, spastic paraplegia was observed in 17 F_1 and in 7 F_2 offspring; this condition was not observed in the parents or in the F_1 and F_2 control groups. Since at least some effects were observed at all dosage levels, the lowest dose at which these effects may be observed is not known. Another unknown factor is whether the levels which produced effects in the study are substantially higher than those "to which humans can reasonably be anticipated to be exposed." In the absence of a level at which there was no demonstrated effect and thus no basis for choice of an "ample margin of safety," it must be presumed that current exposure may be sufficient to produce reproductive and fetotoxic effects in humans.

Dr. Courtney reviewed the study and concluded that the researchers' findings were valid indicators of lindane's adverse effects on normal reproductive functions. Dr. Courtney, noted, however, that not all details of the experimental design were specified and that this limited an analysis of the data. Despite the reporting limitations, Dr. Courtney validated the researchers' findings that gamma-BHC did significantly effect the duration of pregnancy and did decrease the fertility index. Moreover, Dr. Courtney stated that there was a progressive increase in the observed mortality rate of the three generations due to a feto-toxic and/or reproductive effect. Dr. Courtney also noted that the observation of paraplegia in the F_1 and F_2 generation "was probably quite real." However, contrary to Petreschu's conclusions, Dr. Courtney concluded that observations relating to birth defects and tumor incidence were insignificant.

Dr. Courtney contended that the Naishtein reproductive effects rat study (Appendix CC) did not contain sufficient data, as reported, to validate the researchers' conclusions.

B. Acute Toxicity

40 CFR 162.11(a)(3)(i) sets forth several criteria which require that a rebuttable presumption arise because of the acute toxicity of the pesticide to humans and domestic animals [Section 162.11(a)(3)(i)(A)], and to wildlife [Section 162.11(a)(3)(i)(B)]. The Working Group is not aware of any registrations for products containing BHC which meet or exceed these criteria. However, during the rebuttal procedure, all labels for products containing BHC will be reviewed to determine if a potential for adverse effects from acute toxicity exists.

BHC: Position Document 1
List of Appendices

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